

#41508
8/21/02

CONSTRUCTION OF ADCRE VECTORS

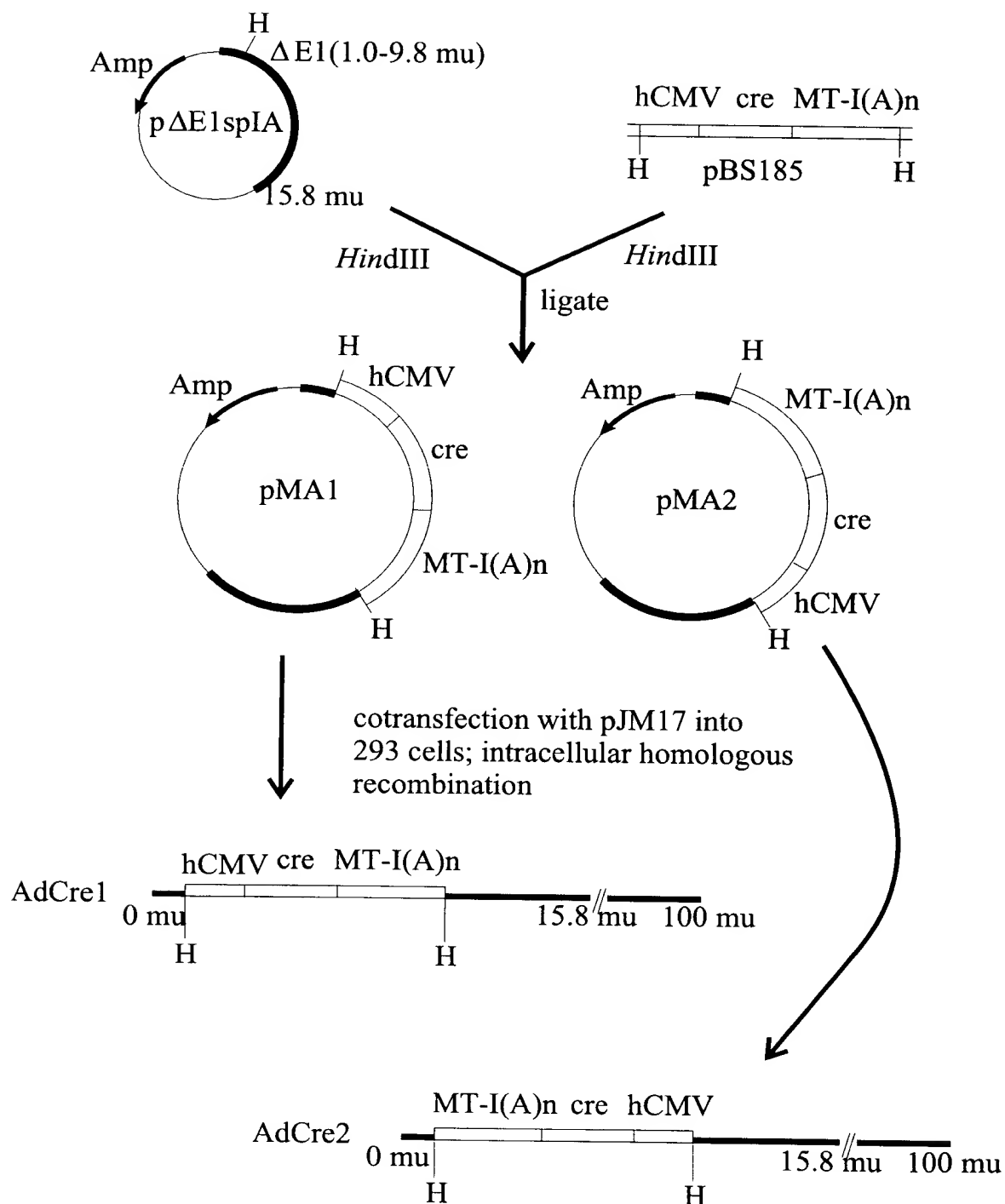


FIGURE 1

EXPRESSION OF CRE RECOMBINASE IN CELLS INFECTED WITH ADCRE

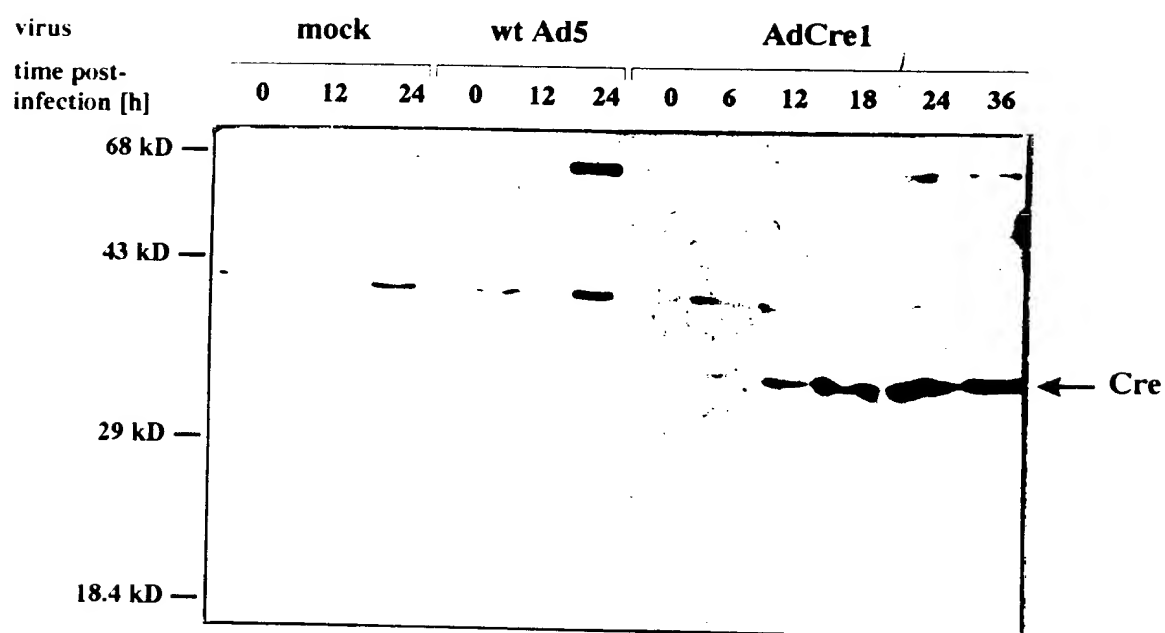


FIGURE 2

CONSTRUCTION OF AD VECTORS EXPRESSING LUCIFERASE UNDER CONTROL OF A MOLECULAR SWITCH

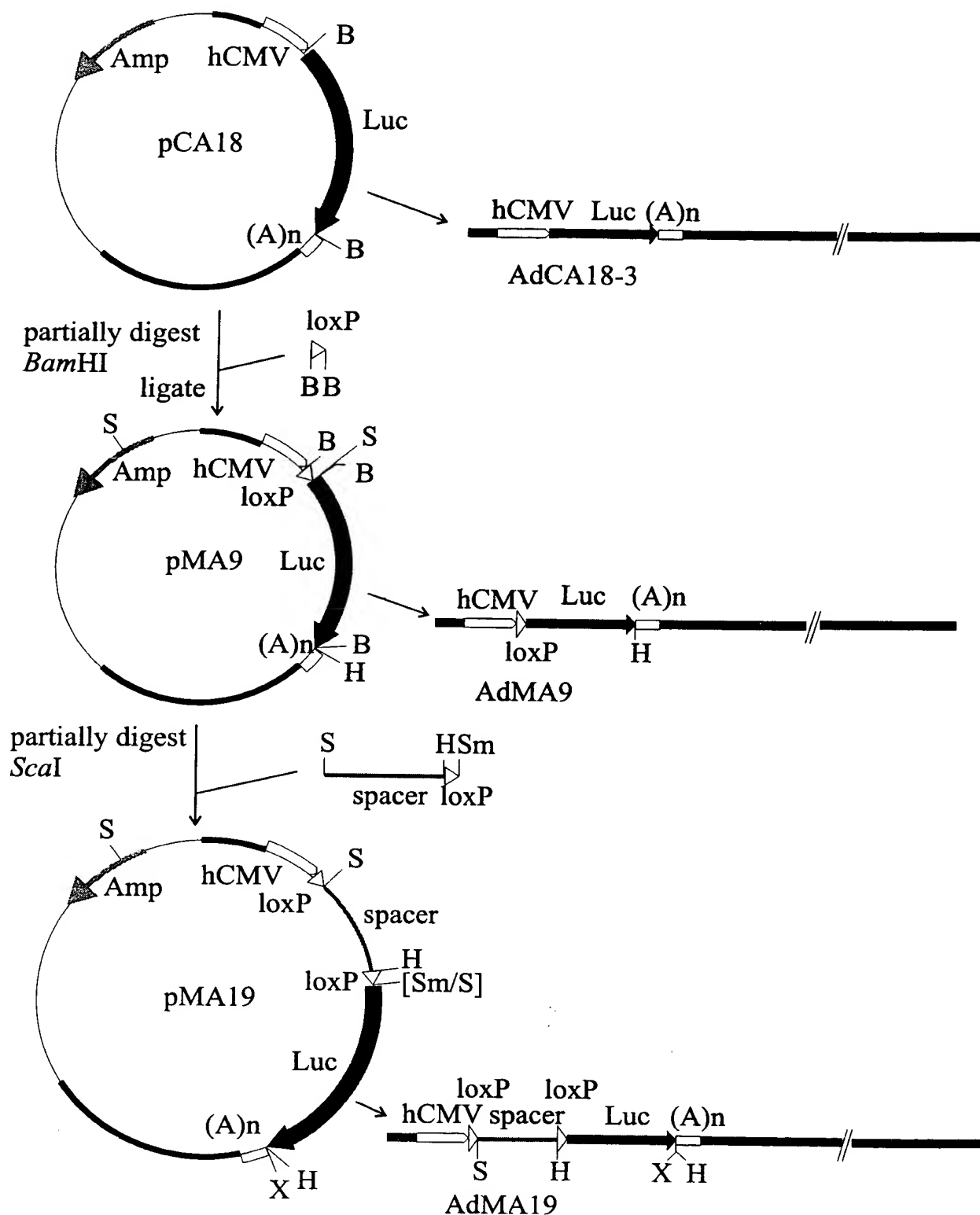


FIGURE 3-1

CONSTRUCTION OF AD VECTORS EXPRESSING LUCIFERASE UNDER CONTROL OF A MOLECULAR SWITCH

SacI

loxP

...TGG...GAGCTC...TCAGAT...CCT...GGATCCA[ATAACTTCGTATAGCATACATTATACGAAGTTATA]
-299 -19 +1 69 95

ScaI

→

→

→

HindIII

AGTACTCAA...AAAATG...TAA...GAAATG...TGA...ATCATG...TGA...AAGCTTGGGCTGCAG...
391 409 425 506 594 672 1355

loxP

[SmaI/ScaI]

[ATAACTTCGTATAGCATACATTATACGAAGTTAT]TAAGGGTTCCGGATCCCCACTGAATTCGGATCC...
1383 1449

→ Luciferase

AAAATGGAA...
1472

FIGURE 3-2

EXCISION OF SEQUENCES FROM ADMA19 IN CELLS COINFECTED WITH ADMA19 AND ADCRE

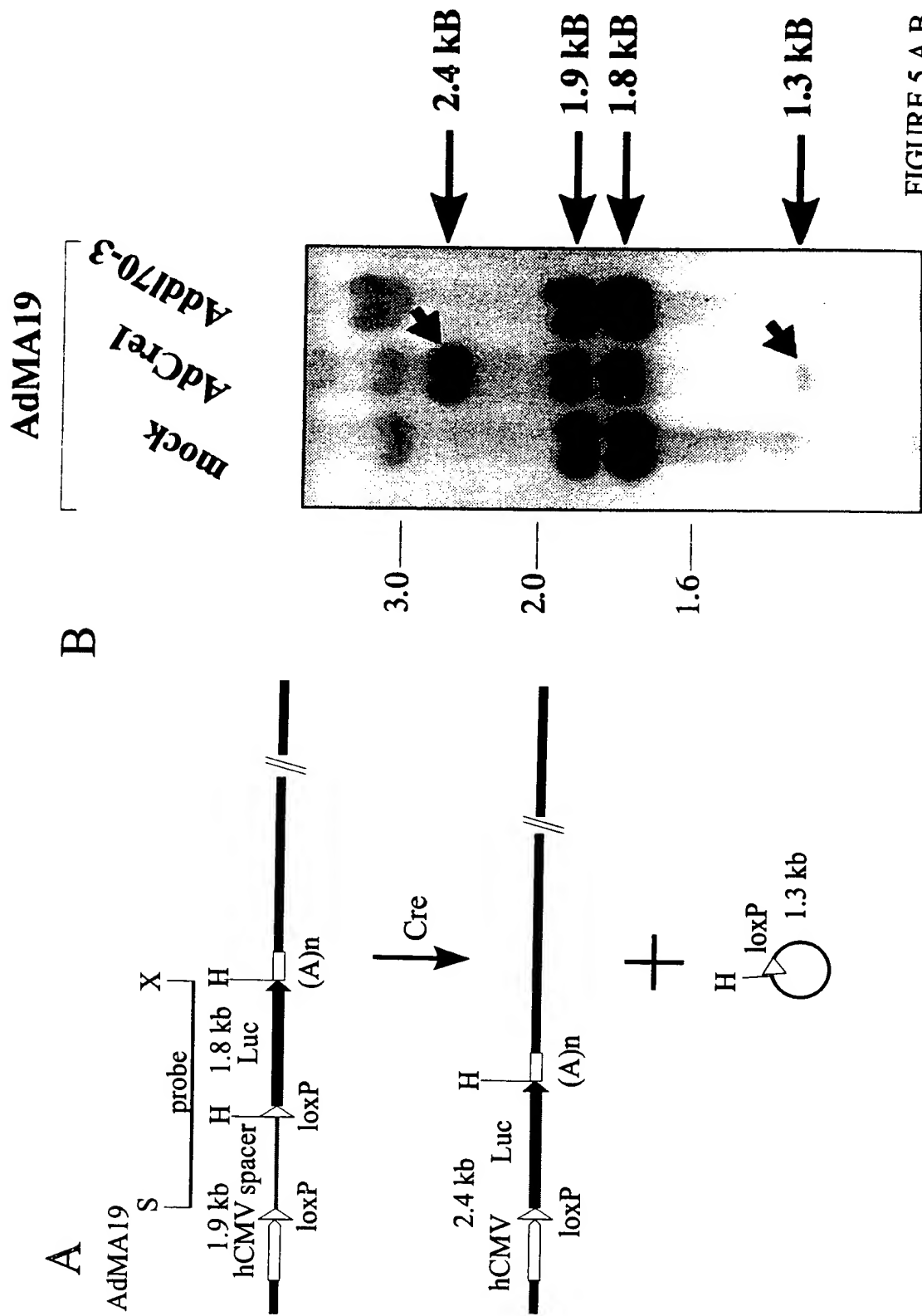
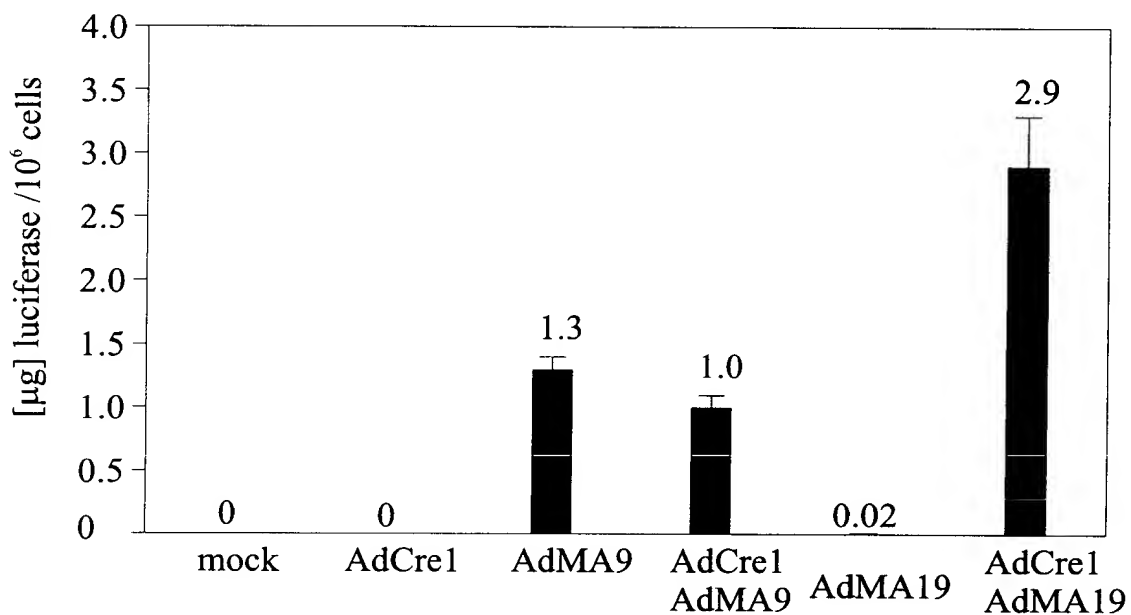


FIGURE 5 A,B

EXPRESSION OF LUCIFERASE IN AD VECTOR INFECTED CELLS

Expt. 1



Expt. 2

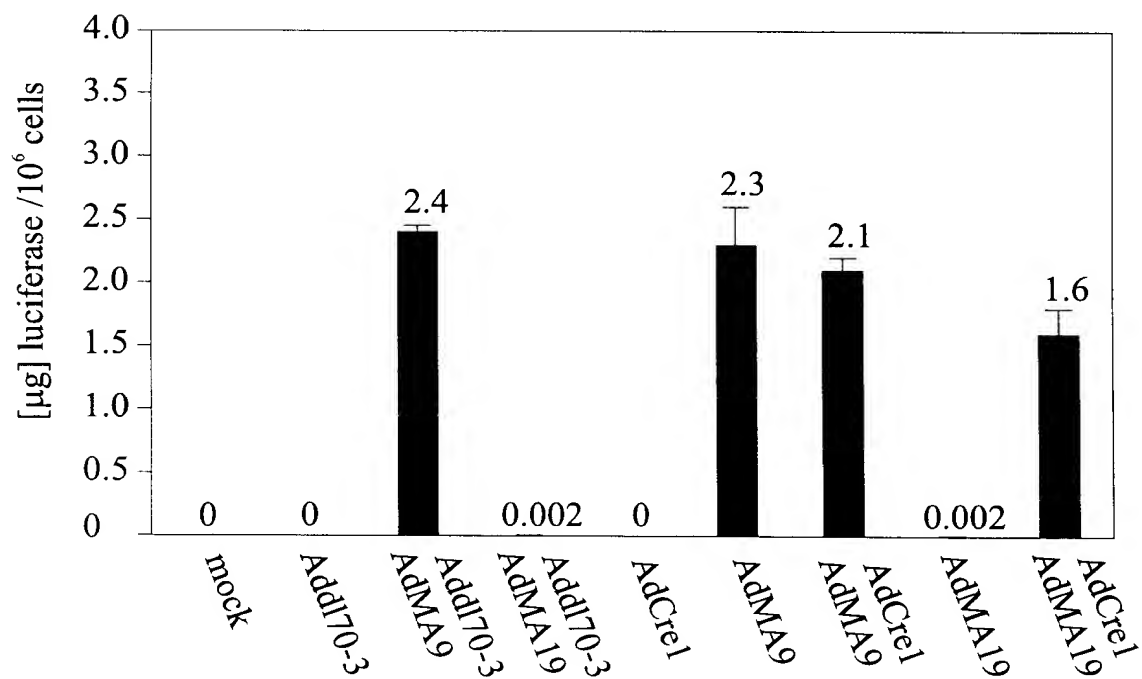
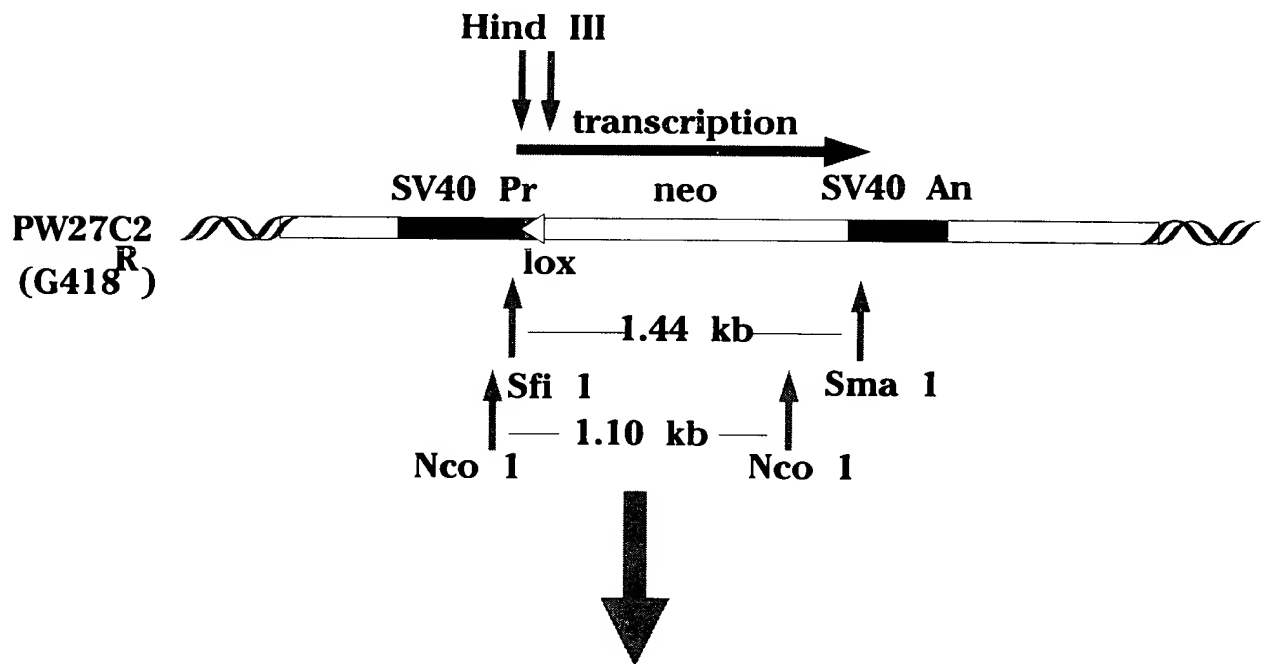


FIGURE 5C

STRUCTURE OF INTEGRATED SEQUENCES IN CELL LINE PW27C2



EXPRESSION OF NEO RESULTING IN G418 RESISTANCE

FIGURE 6A

X4

SOUTHERN BLOT HYBRIDIZATION ANALYSIS OF CELL LINES DERIVED BY TRANSFORMATION OF HT1080 CELLS WITH PBS74

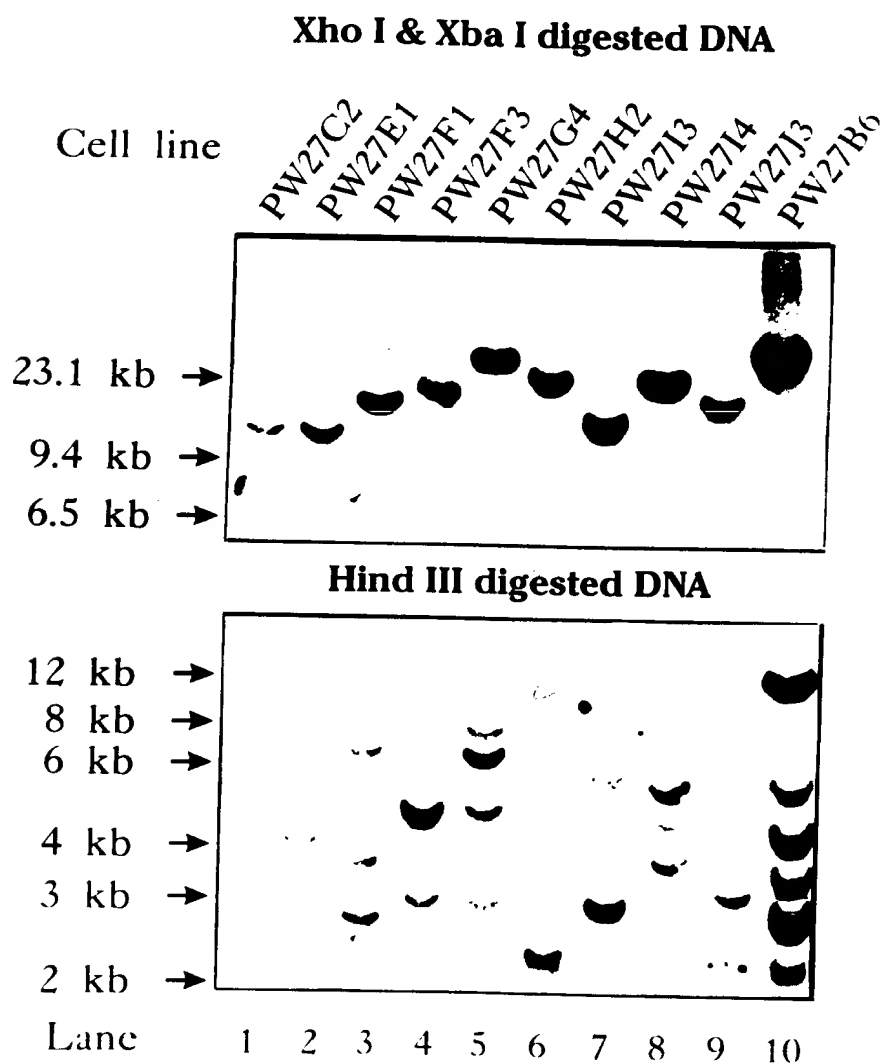


FIGURE 6B

SOUTHERN BLOT HYBRIDIZATION ANALYSIS OF DNA FROM CELL LINES INFECTED WITH ADCRE

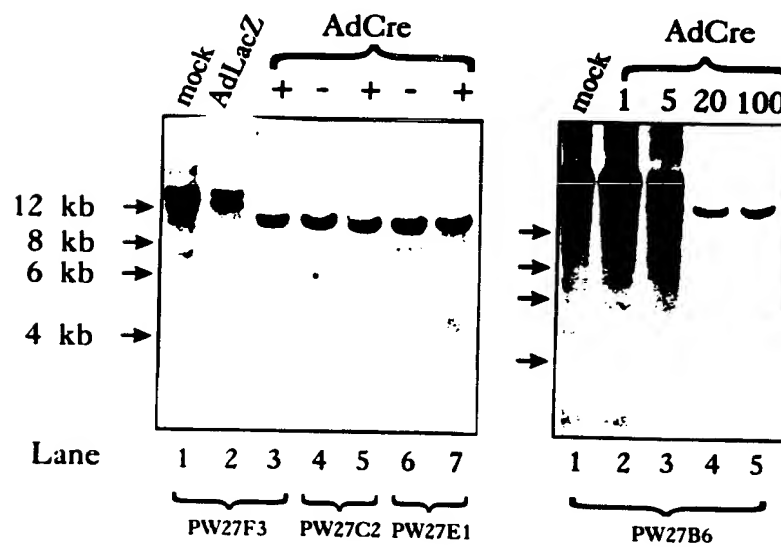


FIGURE 6C

transcription

PW27C2 (G418^R)

SV40Pr neo SV40An

lox

1.44 kb

Sfi 1

1.10 kb

Nco 1

Nco 1

Sma 1

AdCre

AP^r

SV40An

pPW1 6.07 kb

lox

HisD

Cre mediated recombination

neo

PW27C2

lox

1.44 kb

Sfi 1

1.10 kb

Nco 1

Nco 1

Sma 1

SV40An

AP^r

pPW1 6.07 kb

lox

HisD

PW-HC2 (his^R) transcription

lox

SV40Pr hisD SV40An

1.49 kb

Sfi 1

Sma 1

6.07 kb

7.17 kb

Nco 1

Nco 1

Sma 1

SV40An

Expression of hisD resulting in Histidinol resistance

FIGURE 7A

SOUTHERN BLOT HYBRIDIZATION ANALYSIS OF CELL LINES DERIVED BY CRE MEDIATED INTEGRATION OF pPW1

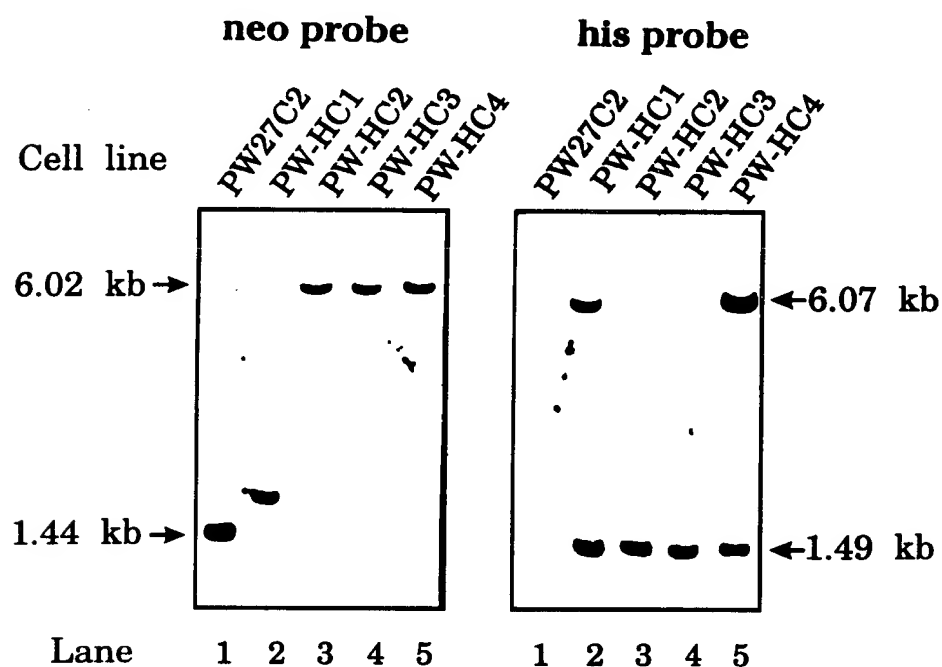
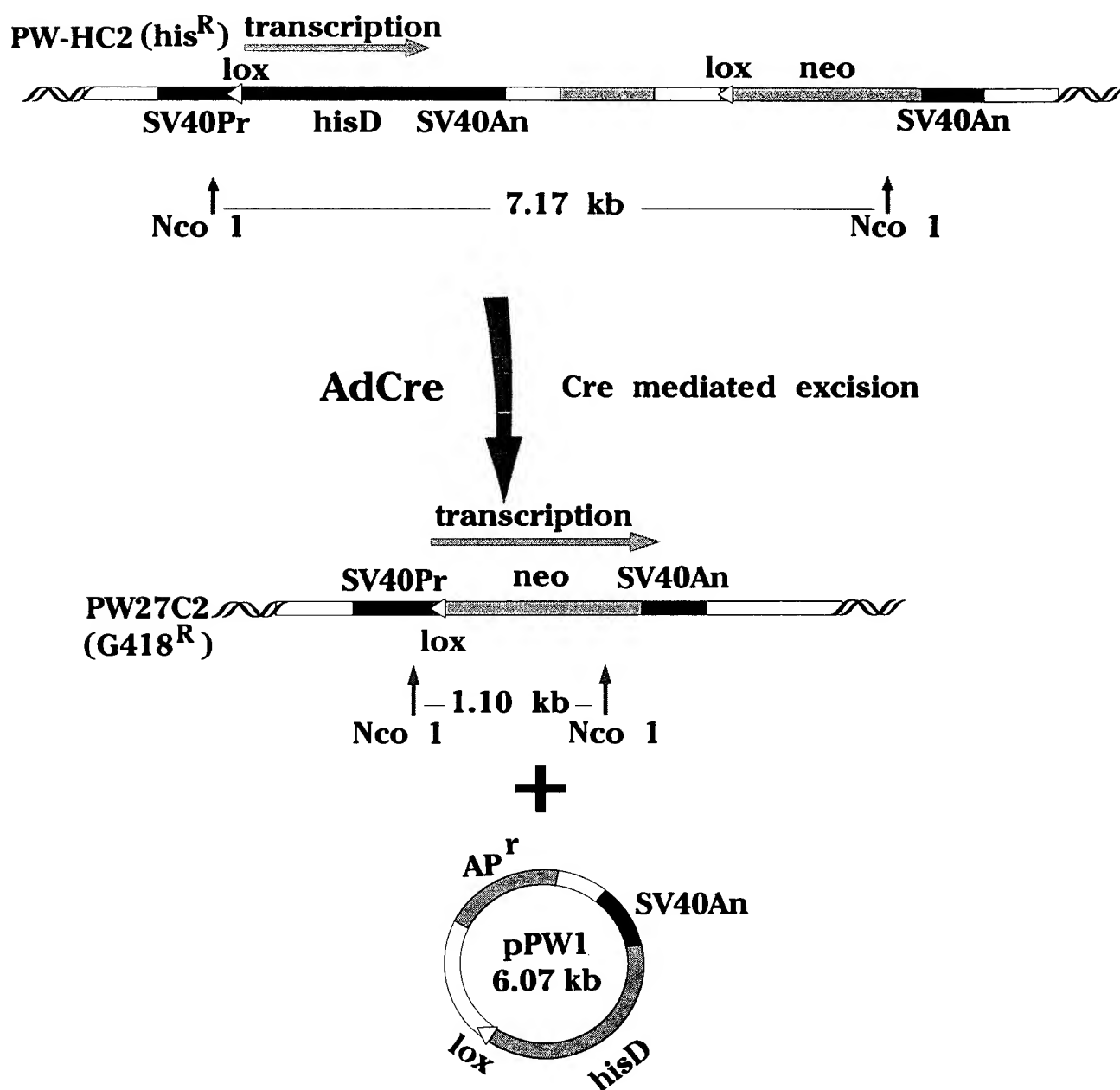


FIGURE 7B

CRE-MEDIATED EXCISION OF DNA CONTAINING hisD SEQUENCES FLANKED BY LOX SITES



**Loss of hisD expression (Histidinol sensitive)
Gain of neo expression (G418 resistant)**

FIGURE 8A

Conversion of HisD resistant cells to
G418 resistant cells by infection with AdCre

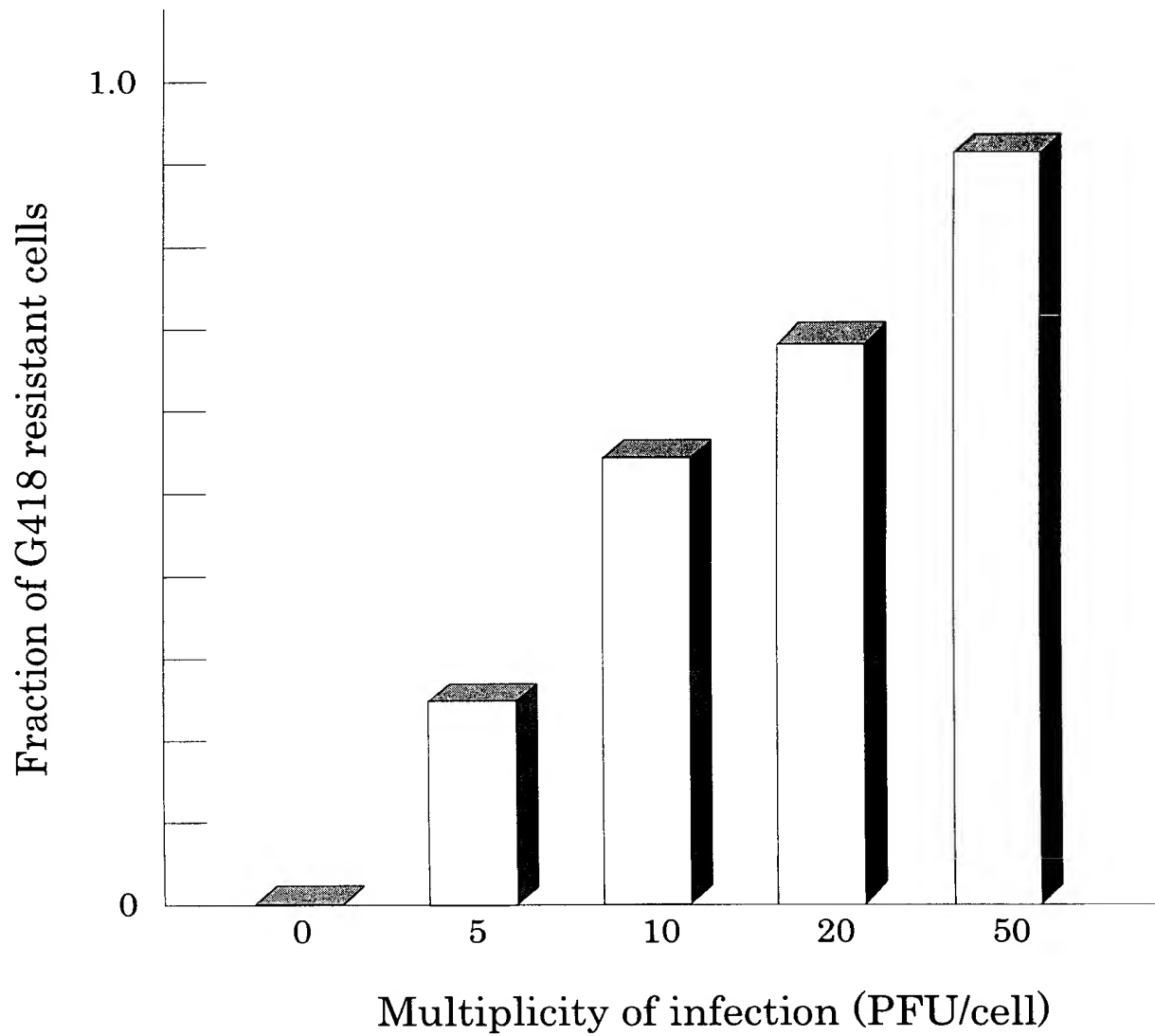
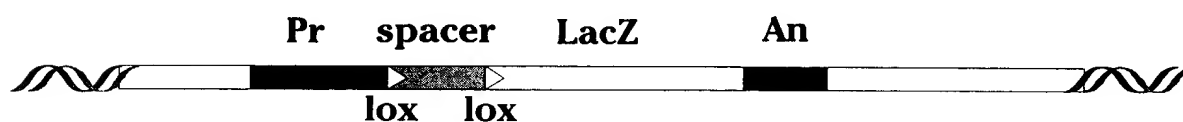


FIGURE 8B

IN VIVO GENE EXPRESSION CONTROLLED BY A MOLECULAR SWITCH



TRANSGENICS CONTAINING GENES CONTROLLED BY A MOLECULAR SWITCH



EXPRESSION OF β -Gal, Rb, P53, Neu ETC.

Figure 9